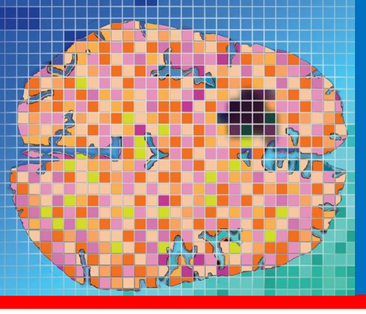

Oxidative Phosphorylation (OXPHOS) Modulation of Immune Response in Melanoma

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Introduction

- Melanoma is the rarest yet most aggressive of the common forms of skin cancer
- Melanoma brain metastases (MBMs) are a leading cause of morbidity and mortality for patients with advanced melanoma
- Previous studies have implicated oxidative phosphorylation (OXPHOS) in the pathogenesis of MBM and in suppression of the anti-tumor immune response
- Clinically, MBMs with high OXPHOS had decreased sensitivity to immunotherapy and targeted gene therapy
- However, the role of OXPHOS in modulating tumor immunity in MBM remains unclear

Hypothesis

We hypothesize that OXPHOS suppresses the immune response in MBMs.

Aim

To better understand the role of OXPHOS in MBM pathogenesis and immunosuppression, we investigated the metabolic and immunologic effects of pharmacological OXPHOS inhibitors using IPN-60090 and IACS-010759 on D4M melanoma cells.

Methods and Materials

Fluorescence Tagging: D4M murine melanoma cells were cultured and stably transfected with lentiviral particles co-expressing firefly luciferase and RFP, and blasticidin antibiotic resistance (GenTarget)

Selection: D4M sensitivity to blasticidin (Bsd) treatment (0.1 µg/mL – 1000 µg/mL) was evaluated by Cell Titer Blue (CTB) assay. Transfected D4M cells underwent dual-selection with blasticidin antibiotic treatment (100 µg/mL) and FACS

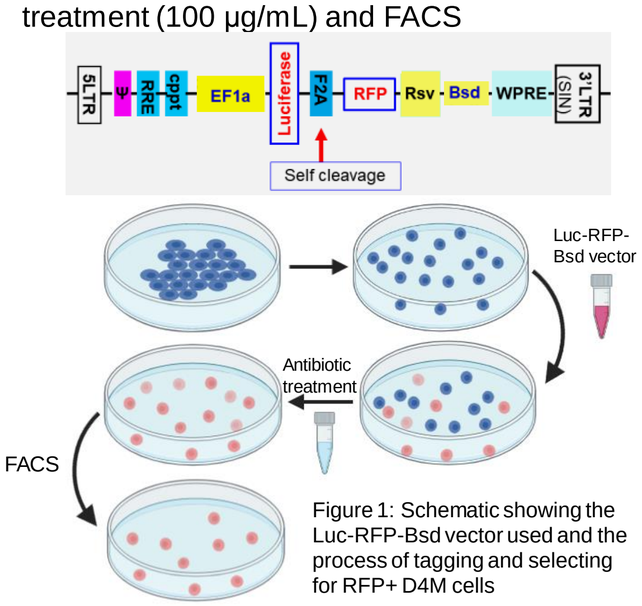


Figure 1: Schematic showing the Luc-RFP-Bsd vector used and the process of tagging and selecting for RFP+ D4M cells

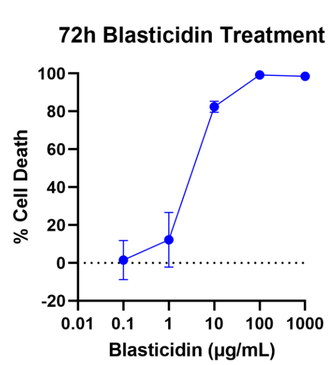
Drug Treatments: Cells were treated with complete media, DMSO, and 0.1 - 5 µM of IPN-60090 (glutaminase inhibitor) and IACS-010759 (mitochondria complex I inhibitor).

Cellular Metabolism Analysis: The MitoStress Test was run on Seahorse 96-well XF Analyzer to measure oxygen consumption rate (OCR) of D4M cells 24h post-drug treatment

Cytokine Analysis: Cell media supernatant was collected at 24h post-treatment with 1 µM IACS-010759. Cytokine array analysis was performed using the Mesoscale U-PLEX platform to assess the effect of OXPHOS inhibition on D4M melanoma secretion of cytokines and chemokines involved in immune system regulation

Results

D4M Blasticidin Sensitivity



	µg/mL Bsd
IC50	4
IC90	25
IC99	100

Figure 2: D4M cell vitality after blasticidin antibiotic treatment analyzed by CTB Assay. Dots represent mean of replicates (n=4) and lines represent SEM.

Fluorescence Tagging of D4M Cells

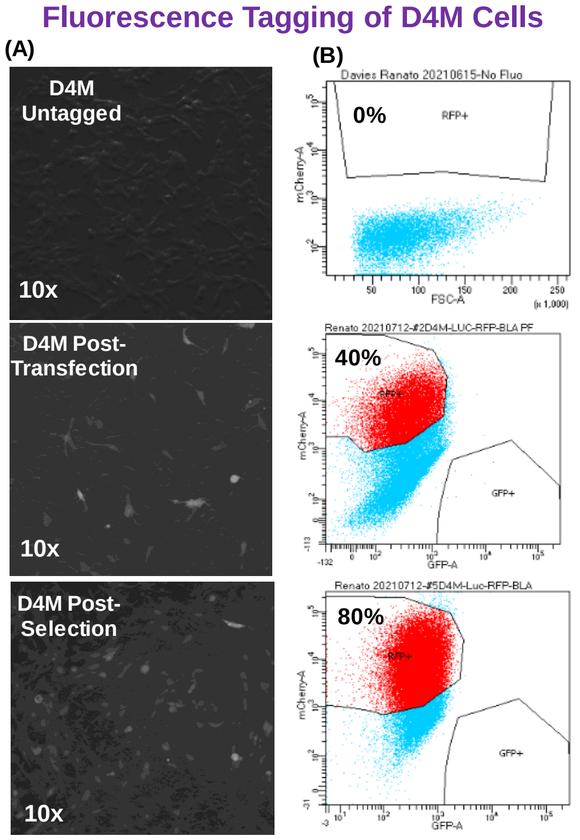


Figure 3: **(A)** Fluorescent microscopy images of D4M cells before and after transfection and selection with blasticidin and FACS. **(B)** Flow cytometry indicating increasing percentage of RFP+ D4M cells.

Results (continued)

Metabolic Effect of OXHPOS Inhibitors on D4M Cells

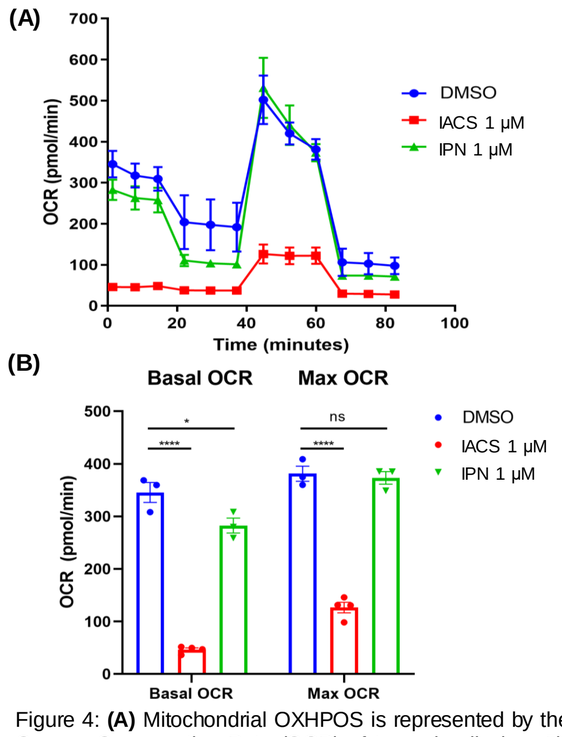


Figure 4: **(A)** Mitochondrial OXHPOS is represented by the Oxygen Consumption Rate (OCR) of treated cells (n = 5). **(B)** Treatment with 1 uM IACS-010759 and IPN-60090 decreased basal OCR by 86.6% (p<0.0001) and 18.2% (p = 0.056) respectively, and IACS decreased the maximum OCR level by 66.8% (p<0.0001). Dots represent individual replicates (n=3) and lines represent SEM.

Immune Effect of IACS-010759 on D4M Cells

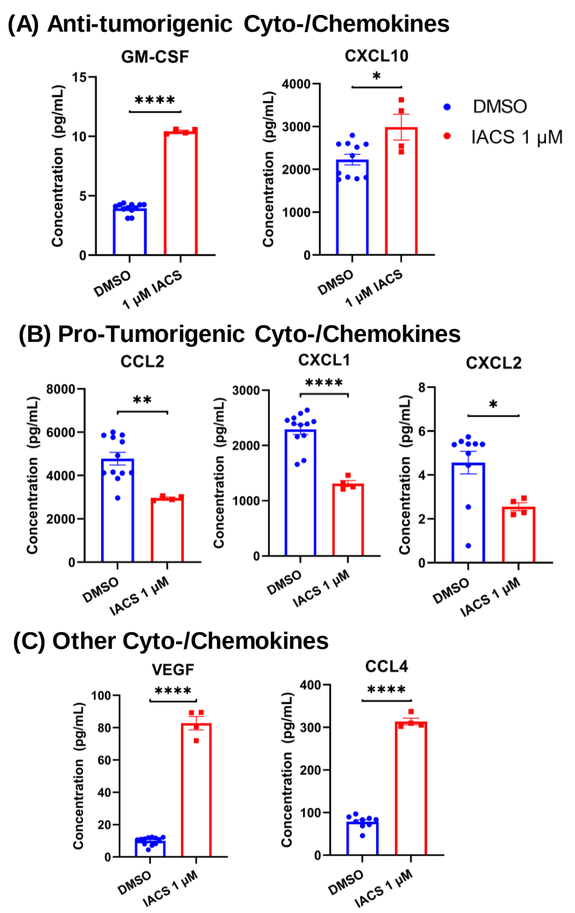


Figure 5: OXHPOS inhibition: **(A)** Increased expression of anti-tumorigenic GM-CSF (p<0.0001) and CXCL10 (p=0.0144), **(B)** Decreased secretion of pro-tumorigenic CCL2 (p=0.0035), CXCL1 (p<0.0001), and CXCL2 (p=0.034), **(C)** Highly increased expression of VEGF (p<0.0001) and CCL4 (p<0.0001).

OXPHOS Modules the Tumor Microenvironment

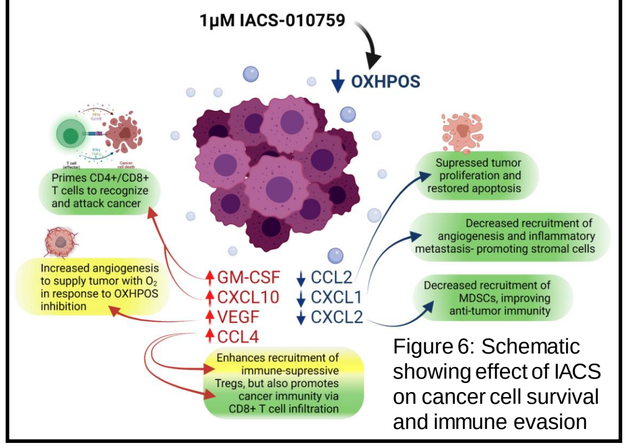


Figure 6: Schematic showing effect of IACS on cancer cell survival and immune evasion

Conclusions

- IACS-010759 and IPN-60090 significantly inhibited OXPHOS levels in D4M cells
- Treatment with OXPHOS inhibitors increased the level of certain immune activators and decreased levels of pro-tumor cytokines secreted by D4M cells
- D4M is more sensitive to inhibition of Complex I than glutaminase inhibition.
- Our results are consistent with growing data showing mitochondrial metabolism and OXPHOS has diverse and critical functions in cancer immune evasion.
- We plan to further assess the long-term effects of OXPHOS inhibition on D4M cytokine expression
- Future studies will examine the impact of the tumor microenvironment in MBM and how it influences the rate and sensitivity of OXPHOS in melanoma cells through co-culture with astrocytes and in animal models of MBM. Animal studies will utilize luciferase expression for in-vivo imaging
- Better understanding of cancer metabolism and its effect on immune response will allow for new, efficient treatments combining metabolic inhibitors with immune-checkpoint therapy.

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